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## Original Paper

# A Phase I Trial of 5-day Chronomodulated Infusion of 5-Fluorouracil and l-Folinic Acid in Patients with Metastatic Colorectal Cancer

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**The aim of this phase I study was to establish the maximum tolerated dose (MTD) of 5-fluorouracil (5-FU), administered as a 5-day chronomodulated infusion in combination with l-folinic acid (FA) to ambulatory metastatic colorectal cancer patients. Consecutive cohorts of 6 patients were given 5-FU and FA infusions from 10.00 p.m. to 10.00 a.m. with peak delivery at 4.00 a.m. by means of a multichannel programmable pump. The FA dose was always the same (150 mg/m$^2$/d). For the first cohort, the 5-FU dose level was 600 mg/m$^2$/d at the first course, escalated by 100 mg/m$^2$ for each subsequent cohort. Intrapatient dose was also escalated by 100 mg/m$^2$ if toxicity was less than grade 2. The courses were repeated every 3 weeks. Thirty-four patients (17 previously treated) received a total of 154 courses. Dose-limiting toxicity consisted of stomatitis and diarrhoea. No significant haematological, cutaneous or cardiac toxicity was encountered. The MTD of 5-FU was reached at the fourth level (first course at 900 mg/m$^2$/d equal to 4500 mg/m$^2$/course) with 5-FU increased to 1100 mg/m$^2$/d (5500 mg/m$^2$/course) in 4 patients. The received 5-FU dose intensity (DI) over the first 3 courses at this level was 1318 mg/m$^2$/week. Thirty-three patients were assessed for response. An objective response was achieved in 1 out of the 13 previously-treated and in 8 out of the 20 previously-untreated patients. The chronomodulated infusion of 5-FU at a dose of 900 mg/m$^2$/d, together with FA at 150 mg/m$^2$/d for 5 days, was safely delivered to outpatients with metastatic colorectal cancer. The low toxic profile and activity of this regimen in previously untreated patients deserves further exploration for the treatment of 5-FU-sensitive tumours.** © **1997 Elsevier Science Ltd.**

**Key words: colorectal cancer, chronotherapy, 5-fluorouracil**
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## INTRODUCTION

CHEMOTHERAPY FOR advanced colorectal cancer is based on the use of 5-fluorouracil (5-FU). The addition of folinic acid (FA) increases the level of thymidylate synthase inhibition and DNA synthesis suppression, improving the objective response rate [1].

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Chronotherapy takes into account the possible benefits of administering drugs at specific times of the day to optimise the therapeutic index. As a consequence, antineoplastic drugs are better tolerated and a higher dose intensity (DI) can be administered.

Fluoropyrimidines (5-FU and FUDR) [2–4], anthracyclines and platinum compounds (cisplatin and oxaliplatin) seem to benefit from selective circadian scheduling, increasing tolerability and DI [5, 6]. The nocturnal administration of 5-FU in cancer patients is based on the following: (1) the

lethal toxicity of 5-FU is 2- to 8-fold less during the daytime in mice when these animals are at rest, as compared to night-time when they are active [7]; (2) continuous 5-FU infusion is associated with greater than 50% circadian changes in drug plasma levels, in both tumour-bearing mice and in patients [8, 9]; (3) less replicative activity has been found in human bone marrow and in the oral and rectal mucosa during the early night hours compared to daytime [10–12]; and (4) the activity of dehydropyrimidine dihydrogenase (DPD), an enzyme involved in 5-FU catabolism, is subject to circadian rhythms in laboratory animals, in healthy human subjects and in cancer patients [13–15]. Opposite rhythms were found in mice or rats in terms of the enzymatic activity involved in the generation of intracellular cytotoxic forms of 5-FU (FUMP and FdUMP) and their incorporation into DNA.

On the basis of these considerations, a chronomodulated infusion of 5-FU, FA and oxaliplatin (L-OHP), a new non-nephrotoxic cisplatin analogue, was designed and has shown a 58% objective response rate in patients with advanced colorectal cancer, regardless of prior 5-FU exposure [16]. These results have been confirmed in two consecutive multicentre randomised phase III trials, in which the chronomodulated infusion of 5-FU, FA and L-OHP was much less toxic and more active than the flat delivery [17, 18]. This latter drug displayed an objective antitumour activity in 3 out of 29 (10%) heavily pretreated patients with advanced colorectal cancer [19]. Therefore, both L-OHP and the chronomodulated delivery of all three drugs contributed to increasing the activity of this regimen at the doses employed.

However, neither the maximum tolerated dose (MTD) of chronomodulated 5-FU and l-FA infusion nor its antitumour efficacy has been established. The aim of this study was, therefore, to define the MTD of this infusion in ambulatory metastatic colorectal cancer patients.

## PATIENTS AND METHODS

Admission criteria included biopsy-proven carcinoma, measurable recurrent or metastatic adenocarcinoma of the colon or rectum and a life expectancy of more than 1 month. Exclusion criteria were: age above 75 years, a performance status (PS) greater than 2 (WHO); surgically resectable metastases and more than one chemotherapy line for metastatic disease.

Previous radiotherapy was not an exclusion criteria. Patients with inadequate bone marrow reserve (HB < 8 g/dl or leukocyte count <3000 $mm^3$, platelet count 100000 $mm^3$) and liver function tests (transaminases >1.5 normal) as well as renal dysfunction (creatinine >1.5 mg/dl) were also excluded.

Eligible patients underwent a complete clinical history and physical examination and surgical placement of a totally implanted double-venous access port (Port-A-Cath, Pharmacia, Sweden). Weight and height, complete blood cell counts and serum bilirubin, creatinine, ionogram, calcium, magnesium, total protein, alkaline phosphatase, serum liver transaminase, CEA and CA19-9 levels were also determined. The patients were submitted to chest x-rays, abdominopelvic computed tomographic (CT) scanning and colonoscopy one month before beginning the treatment.

*Table 1. Study design: 5-FU doses*

| Level | 1st course | 2nd course | 3rd course |
|---|---|---|---|
| I | 600 | 700 | 800 |
| II | 700 | 800 | 900 |
| III | 800 | 900 | 1000 |
| IV | 900 | 1000 | 1100 |
| V | 1000 | 1100 | 1200 |

5-FU: mg/$m^2$ i.v. for 5 days, time-modulated infusion.
All the patients also received L-folinic acid 150 mg/$m^2$ i.v.for 5 days concomitantly with 5-FU. The courses were repeated every 3 weeks. Groups of at least 6 patients were included at each dose level.

The protocol was approved by the local ethics committee and oral informed consent was obtained from all the patients.

*Study design*

Six patients were included in the first course for each dose level (Table 1). An initial 5-FU dose of 600 mg/$m^2$/d was chosen, as being the first level used in the multicentre phase III trials with the 5-FU-FA-L-OHP regimen [18]. In the present study, the l-form of FA was concurrently infused with 5-FU at a fixed dose of 150 mg/$m^2$/d, corresponding to 300 mg/$m^2$/d of the racemic form of FA used in the three-drug chronomodulated regimen [20]. The 5-FU dose was increased by 100 mg/$m^2$/d in consecutive cohorts of 6 patients until a toxic level was reached. In addition, intrapatient dose escalation was made at the second and/or third course in the absence of grade 2 or greater diarrhoea, mucositis or haematological suppression.

The dose remained fixed in the event of grade 2 toxicity but was reduced by 100 mg/$m^2$/d in the presence of grade 3 toxicity. Patients with grade 4 toxicity went off study. The dose level remained fixed after the third course. The toxic level was defined as that in which 3 out of 6 patients had grade 3 or 4 toxicity after the first course. The MTD level was defined as that immediately below the toxic level. The received 5-FU DI was computed according to Hryniuk at each dose level in the first three courses of therapy [21].

Therapy was administered to out-patients by means of a multichannel programmable pump (Intelliject, Aguettant, Lyon, France). Two 30 ml reservoirs were filled with 5-FU at a concentration of 50 mg/ml and the other two with FA at a concentration of 20 mg/ml. The drugs were automatically administered from 10.00 p.m. to 10.00 a.m. with a peak at 4.00 a.m. for 5 consecutive days every 3 weeks. No drug was infused between 10.00 a.m. to 10.00 p.m. (Figure 1).

*Toxicity assessment*

Clinical examinations and all biological determinations were made before each course (on day 21). Toxicity was assessed according to WHO criteria [22]. Metoclopramide was employed as the first-line antiemetic drug which was replaced by ondansetron only in patients experiencing grade 2 or greater nausea and vomiting.

*Evaluation of response*

Response was evaluated after every three courses by means of CT scanning and was defined according to the following criteria: (1) complete response (CR): complete dis-

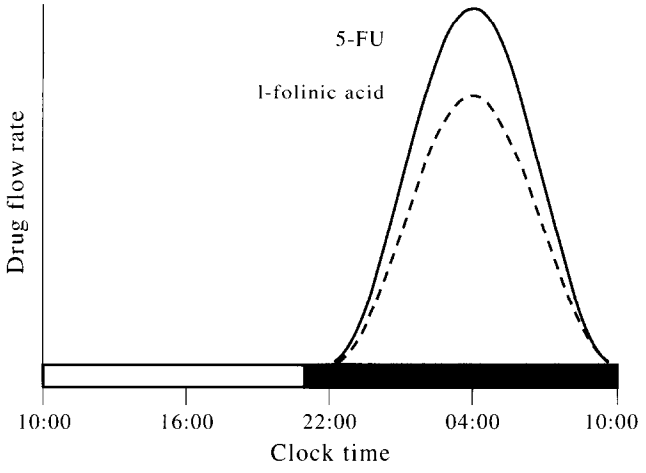


**Figure 1. Drug delivery schedule administered for 5 consecutive days.**

appearance of all symptoms and signs of disease for a minimum of 4 weeks; (2) partial response (PR): a 50% reduction (or more) in the sum of the products of the perpendicular diameters of measurable disease and the appearance of no new areas of disease; (3) stable disease (SD): less than 50% decrease or 25% increase in the described measurements; and (4) progressive disease (PD): a more than 25% increase in the measurements and/or the appearance of new lesions.

The evaluations were made at the Regina Elena Cancer Institute and all CT scans were always examined by the same radiologist, together with a medical oncologist.

*Table 2. Patient characteristics. Inclusion period: April 1992–August 1994*

| | |
|---|---|
| No. of patients | 34 |
| Sex (M/F) | 19/15 |
| Median age | 57 years |
| (range) | (24–75) |
| Primary tumour | |
| colon | 23 |
| rectum | 11 |
| Prior therapy | 17 |
| chemotherapy | 11 |
| radiotherapy | 4 |
| both | 2 |
| Performance status (WHO) | |
| 0–1 | 28 |
| 2 | 6 |
| No. of metastatic sites | |
| 1 | 20 |
| 2 | 12 |
| >2 | 2 |
| Sites involved | |
| liver | 27 |
| abdomen | 14 |
| lung | 5 |
| bone | 3 |
| primary tumour | 7 |
| Tumour markers | |
| CEA >5 ng/ml | 26 |
| CA19-9 > 36 U/ml | 22 |

## RESULTS

*Patient characteristics*

Thirty-four patients entered the study. Seventeen had previously received chemotherapy (Table 2). One hundred and fifty-four courses of therapy were given and all were assessed for toxicity. The patients received a median number of three courses (from 1 to 10).

*5-FU dose*

The 5-FU dose at the first course ranged from 600 mg/m$^2$/d to 1000 mg/m$^2$/d. The toxic level was the fifth one (5-FU dose of 1000 mg/m$^2$/d at the first course), mucositis and diarrhoea being dose limiting. One patient had grade 4 mucositis and concurrent grade 3 mucositis and diarrhoea were observed in another. A third patient displayed grade 3 mucositis and a fourth had grade 3 diarrhoea. Accrual at this level was, therefore, closed.

The fourth level was considered as the MTD, the initial 5-FU dose being 900 mg/m$^2$/d with subsequent increments up to 1000, then 1100 mg/m$^2$/d. Five additional patients were treated at this level without experiencing any grade 3 toxic symptoms. Of the 11 patients registered at the MTD level, 4 discontinued treatment for PD before the third course, 1 had a reduced 5-FU dose due to toxicity, 1 continued 5-FU at 900 mg/m$^2$/d for the first three courses and 5 tolerated dose escalation up to 1000 mg/m$^2$/d for 1 patient and up to 1100 mg/m$^2$/d for 4 patients.

*Major events and protocol compliance*

No toxic deaths were encountered. Eight patients withdrew from the study before the third course. Early PD was observed in 7 patients. One patient, previously suffering from angina pectoris, exhibited atrial flutter after the first course at a 5-FU dose level of 800 mg/m$^2$/d. This patient developed the same symptom after receiving different cytotoxic drugs (cisplatin and FUDR), so no firm relationship with 5-FU could be established. For this reason, 1 additional patient was included at the third level. Therapy was delayed by 7 or more days in 7 patients: for toxic symptoms in 4 and for occlusion of a single lumen side-port in 3. A double-lumen side-port was then implanted in these patients.

Twenty patients experienced grade 2 toxicity; only 6/34 (18%) displayed grade 3 or 4 toxicity, with some patients experiencing more than one toxic effect. Toxic effects were present in only 18/154 courses (11.7%) (Table 3). The interpretation of nausea and vomiting should take into account that anti-$HT_3$ anti-emetics were employed only after grade 2 vomiting. No neurological, renal or hepatic toxicity was observed. Grade 2 haematological toxicity (leucopenia) was observed in only 1 patient in association with grade 4 mucositis and diarrhoea after a course of 1000 mg/m$^2$/d 5-FU. A DPD deficiency was suspected, but this test

*Table 3. WHO non-haematologic grade 3–4 toxicity in 34 patients (154 courses of therapy)*

| Grade 3–4 toxicity | Patients (%) | Courses (%) |
|---|---|---|
| Mucositis | 5 (15) | 7 (4.5) |
| Diarrhoea | 2 (6) | 4 (2.5) |
| Vomiting | 2 (6) | 4 (2.5) |
| Hand–Foot syndrome | 1 (3) | 3 (2.0) |

*Table 4. 5-FU dose intensity (DI) over 3 courses and response to treatment*

| | All patients | | Previous 5-FU: Yes | | Previous 5-FU: No | |
|---|---|---|---|---|---|---|
| Level | DI (mg/m$^2$/week) | No. of responses | DI (mg/m$^2$/week) | No. of responses | DI (mg/m$^2$/week) | No. of responses |
| I | 1027 | 0/6 | 1118 | 0/3 | 937 | 0/3 |
| II | 1256 | 1/6 | 1273 | 0/4 | 1228 | 1/2 |
| III | 1146 | 3/6 | – | 0/0 | 1146 | 3/6 |
| IV | 1353 | 2/6 | 1145 | 0/2 | 1353 | 2/4 |
| V | 1193 | 1/4 | – | 0/0 | 1193 | 1/4 |
| IV$^{bis}$ MTD | 1284 | 2/5 | 1216 | 1/4 | 1555 | 1/1 |
| | | 9/33 (27%) | | 1/13 (8%) | | 8/20 (40%) |

was not conducted. Two patients, treated in the first course with 900 mg/m$^2$/d and 1000 mg/m$^2$/d 5-FU, respectively, experienced a syndrome related to the infusion time. This consisted of cramp-like abdominal pain in the epigastric area and a feeling of great malaise, dizziness and nausea occurring close to 5.00 a.m., soon after 5-FU peak delivery. In one case, the EKG alterations were compatible with cardiac ischaemia. Treatment was withdrawn in both these patients because of these severe side-effects.

*Dose intensity and response*

The received 5-FU DI over the first three courses was calculated at each level (Table 4). This ranged from 1027 mg/m$^2$/week at the first level to 1353 mg/m$^2$/week at the fourth level. The received 5-FU DI ranged from 65% of the theoretical DI at the toxic level to 94% at the second level. The 11 patients treated at the MTD received a 5-FU DI of 1318 mg/m$^2$/week, 80% of that projected.

Thirty-three of the 34 patients were evaluated for response, the patient suffering from atrial flutter being unassessable. Nine patients achieved a PR (27%) and 2 displayed a minor response. Seven patients had SD and 15 presented PD. Of the 13 patients previously treated with 5-FU, only 1 responded (8%). Eight of the 20 untreated patients achieved a PR (40%). One out of 12 responses (8%) was obtained in the patients treated at the first two levels (average 5-FU DI: 1141 mg/m$^2$/week), while 8 out of 21 (38%) corresponded to the higher levels (average 5-FU DI: 1244 mg/m$^2$/week). In the untreated patients, one out of 5 responses was obtained at the first two levels (average 5-FU DI: 1082 mg/m$^2$/week) and 7 out of 15 at the higher levels (average 5-FU DI: 1311 mg/m$^2$/week).

## DISCUSSION

Chronotherapy is based on biological rhythms, aimed at achieving better activity and tolerability of anticancer drugs. This strategy should allow for a high DI and consequently greater efficacy, as postulated by Hryniuk in patients with metastatic colorectal cancer [21]. The availability of portable multichannel pumps has rendered these treatment modalities feasible even in out-patients.

The present study was aimed at defining the MTD of combined 5-day chronomodulated 5-FU and FA according to the modality of infusion designed by Lévi [17]. The MTD corresponded to 900 mg/m$^2$/d of 5-FU (4500 mg/m$^2$/course) and 150 mg/m$^2$/d of l-FA (750 mg/m$^2$/course). The received 5-FU DI in the 11 patients treated at this level was 1318 mg/m$^2$/week over the first 3 courses. Three other phase I trials have evaluated the tolerability and the recommended doses of the 5-FU and FA chronomodulated infusion (Table 5) [23–25]. Even though the aim of this study was only to determine the MTD with the proposed schedule of 5-FU and l-FA infusion, an indirect comparison with the other 'chrono' infusions could be attempted. The present regimen clearly permits delivering the highest 5-FU DI, together with high-dose l-FA. Further randomised studies may be needed to define the optimal chronomodulated regimen with regard to the relevance of such differences for toxicity and antitumour activity.

Severe toxicity (grade 3 or 4) was observed in all 4 patients treated with 1000 mg/m$^2$/d of 5-FU at the first course. However, the incidence of grade 3–4 toxic effects over the total 154 courses was extremely low, being only 4.5% for mucositis and 2.5% for diarrhoea. These rates are much lower than those observed in the phase III trials

*Table 5. Summary of reported phase I trials of chronomodulated 5-FU-FA infusions*

| | MTD mg/m$^2$/d | | | DI mg/m$^2$/week | |
|---|---|---|---|---|---|
| | 5-FU | FA | Infusion duration/interval (d) | 5-FU | Pump |
| Bjarnason '93 [23] | | | | | |
| 14 days every 2 weeks | | | | | |
| peak flow: 9.00–10.00 p.m. | 250 | 20 | 14/14 | 875 | Vivus |
| Adler '94 [24] | | | | | |
| 5 days every 4 weeks | | | | | |
| peak flow: 12.00 p.m.–7.00 a.m. | 600 | 60 | 5/23 | 625 | Chronomat |
| Falcone '95 [25] | | | | | |
| 14 days every 2 weeks | | | | | |
| peak flow: 4.00–12.00 p.m. | 250 | 20 | 14/14 | 875 | Cadd-Plus |
| Present study | | | | | |
| 5 days every 3 weeks | | | | | |
| peak flow: 4.00 a.m. | 900 | 150 | 5/16 | 1318 | Intelliject |

where 5-FU-FA was combined with l-OHP [18]. Furthermore, 4 out of 11 patients who received a 5-FU dose of 900 mg/m$^2$/d at the first course (MTD), tolerated dose increments up to 1100 mg/m$^2$/d in subsequent courses.

This may be due to an induction of enzymatic activities involved in 5-FU or FA metabolism as a consequence of the first treatment course, as recently proposed [26]. In this latter study, a clear relationship between the 5-FU plasma levels and the objective response was demonstrated.

No pharmacokinetic assays were performed in the present study. However, Metzger and associates [27] examined the pharmacokinetics of 5-FUFA and L-OHP chronomodulated infusion and found that the mean 5-FU plasma levels paralleled the chronomodulated drug delivery programme. The largest interpatient difference in the 5-FU concentrations was less than the interpatient difference observed during the flat 5-FU infusion. Assier and associates [28] also conducted a pharmacokinetic study with the 'chrono' 5-FUFA doses established in the present study for 4 days every 2 weeks, confirming that the 5-FU plasma peak occurred near the peak delivery. An active folinic acid metabolite, 5-methytetrahydrofolate, showed a shift of 2 h later, suggesting that, for optimal potentiation, peak delivery of FA could be earlier.

An improvement of patients' general conditions, as a consequence of the first treatment course, may also enhance tolerability. Thus, the incidence of toxic effects from the standard 5-FU-FA regimen was clearly lower in patients with a good PS compared to those with a poor PS which may be even more significant in chronomodulated regimens.

The restoration or amplification of the circadian rhythmicity in variables such as blood cells or hormones appeared to be an early indicator of response to chemotherapy in patients with advanced breast or ovarian cancer [29]. If confirmed in patients with metastatic colorectal cancer, this might explain why the administration of an appropriate chronomodulated delivery schedule becomes even more tolerated when the patients are in better general condition.

Chollet and associates administered chronomodulated 5-FU and FA for 5 days to 19 previously untreated patients with metastatic colorectal cancer at doses comparable to our first and second dose levels. The lack of severe toxicity at these doses was confirmed and 6 objective responses were obtained (35%) [30]. Although our study was not aimed at evaluating treatment efficacy, 9 objective responses were obtained in 33 assessable patients (27%). One response was achieved in the 13 patients previously treated with 5-FU. This 8% objective response rate is lower than that obtained when L-OHP is added to the chronomodulated FU-FA schedule [31]. In contrast, 8 objective responses were obtained in the 20 previously untreated patients (40%). Most of the responses were achieved at the higher 5-FU dose levels, thus corresponding to higher 5-FU DIs (1311 mg/m$^2$/week versus 1082 mg/m$^2$/week).

Such an apparent relationship between 5-FU DI and response was also documented in a recent phase II trial on the intensified chronomodulated 5-FU-FA-I-OHP regimen [32]. Furthermore, in a randomised comparison of flat versus chronomodulated three-drug delivery, the higher 5-FU DI observed in the chronotherapy arm may well account for the higher response rate achieved with this schedule [18]. However, the chronomodulated infusion of 5-FU is not the only way to increase both the received DI and the activity of this drug. Even higher DIs can be achieved with the weekly 24–48 h of flat 5-FU ± FA infusion [33, 34], yet with apparently greater toxicity than the present chronomodulated schedule [35]. Other active infusional schedules have also been reported [36, 37].

A comparison of this chronomodulated regimen with these different 5-FU infusions should be made to determine the optimal treatment in terms of response rate, toxicity and quality of life.

The low toxic profile of this high-dose regimen also makes it an ideal candidate for fully exploring its antitumour activity with other active drugs such as irinotecan or L-OHP. The present results demonstrate the need to evaluate further the antitumour efficacy of high-dose chronomodulated infusions of 5-FU and FA in patients with previously untreated colorectal cancer, as well as in those with breast or head and neck cancer.

In conclusion, the recommended doses to assess properly the antitumour activity of a 5-day chronomodulated 5-FU and l-FA infusion are 900 mg/m$^2$/d of 5-FU and 150 mg/m$^2$/d of l-FA, each course being repeated after an interval of 16 days (FF5-16). The 5-FU dose may be gradually escalated to 1100 mg/m$^2$/d in the absence of grade 2 or greater toxicity. A phase II trial of this FF5-16 regimen at these recommended doses is ongoing at our institute. In addition, the dose intensity of this schedule was further intensified by 20% through the administration of these doses for 4 days, followed by a 10 day interval (FF4-10) [28]. This intensified chronotherapy schedule is also being tested in a multicentre European study by the International Organization for Cancer Chronotherapy. Both of these trials are conducted in patients with previously untreated advanced colorectal cancer in order to guide the design of randomised comparisons of chronotherapy versus a conventional modality of 5-FUFA infusions at their respective recommended doses.


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